FLUMEQUINE

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ADDENDUM

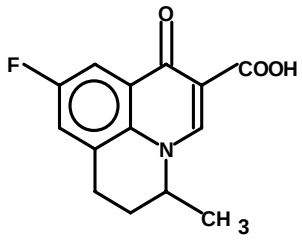
To the monographs prepared by the 48th, 54thand 60th meetings of the Committee and published in the FAO Food and Nutrition Papers 41/10, Rome 1998; 41/13, Rome 2000; and 41/15, Rome 2003

IDENTITY

Chemical name: 9-Fluoro-6,7-dihydro-5-methyl-1-oxo-1H,5H-benzo[ij]quinolizine-2-carboxylic acid

Synonyms: R-802, Apurone

Structural formula:



Molecular formula: $C_{14}H_{12}NFO_3$

Molecular weight: 261.26

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: flumequine

Appearance: white microcrystalline powder

Melting point: 253-255ºC

Solubility: Soluble in aqueous alkaline solutions and alcohol; insoluble in water

Optical rotation: Produced and used as a racemic mixture

BACKGROUND

Flumequine has previously been evaluated by the Committee at the 42nd, 48th, 54th, 60th and 62nd meetings. At its 42nd meeting, the Committee (WHO, 1995) was unable to establish an ADI for flumequine due to the lack of adequate information on the toxicological and microbiological hazards of flumequine and residue data were not provided for evaluation.

The 48th meeting of the Committee (WHO, 1998) established an ADI of 0-30 μg per kg of body weight based on the overall NOEL for hepatotoxicity of 25 mg per kg of body weight per day observed in a 13-week study in CD-1 mice and a safety factor of 1000. MRLs for flumequine of 500 μg/kg for muscle, 1000 μg/kg for liver, 3000 μg/kg for kidney and 1000 μg/kg for fat in cattle, expressed as parent drug, were recommended. Temporary MRLs of 500 μg/kg for muscle, 1000 μg/kg for liver, 3000 μg/kg for kidney and 1000 μg/kg for fat, expressed as parent drug, were recommended in sheep, chickens and pigs. The Committee also recommended a temporary MRL of 500 μg/kg for trout muscle (including normal proportions of skin), expressed as parent drug.

The 54th meeting of the Committee (WHO, 2001) evaluated data from studies conducted with radiolabelled flumequine in pigs, sheep, chickens and trout. Based on these studies, the Committee recommended MRLs of 500 μg/kg for muscle and liver, 3000 μg/kg for kidney and 1000 μg/kg for fat in cattle, pigs, sheep and chickens. The Committee also recommended an MRL of 500 μg/kg for trout muscle, including skin in natural proportions.

The 60th meeting of the Committee (WHO, 2003) evaluated new studies which further elucidated the mechanism of flumequine-induced hepatotoxicity in mice. Based on these studies, the Committee noted the possibility that flumequine induces tumours in the mouse liver by a mechanism that includes genotoxic effects. The Committee therefore withdrew the ADI that had been established at the 48th meeting and withdrew the MRLs that had been recommended at the 54th meeting.

The 62nd meeting of the Committee (WHO, 2004) reconsidered three short-term studies in mice that had been evaluated at the 60th meeting. The Committee also evaluated a new study conducted with flumequine in rat liver cells *in vivo* to assess unscheduled DNA synthesis. The Committee concluded that the available data supported a non-genotoxic, threshold-based mechanism for tumour formation by flumequine in the mouse liver. As a result, the Committee re-established the ADI of 0-30 μg/kg body weight that had originally been established for flumequine at its 48th meeting. The MRLs that had been recommended at its 54th meeting and withdrawn at its 60th meeting were also re-established.

The 62nd meeting of the Committee also recommended a temporary MRL of 500 μg/kg for muscle of black tiger shrimp (*Penaeus monodon*) based on the evaluation made at the 60th meeting. The MRL was temporary and the following information was requested by 2006:

(i) A detailed description of a regulatory method, including its performance characteristics and validation data

The 60th meeting also evaluated residue depletion studies in shrimp. Flumequine was administered to shrimps at a rate of 12 mg/kg of body weight as a single intramuscular injection, or as a single forced oral dose, or as medicated feed for 5 consecutive days. Of the three methods of drug delivery, only medicated feed is practical for large-scale use in shrimp production. Flumequine delivery in feed resulted in the lowest concentration of residues in shrimp muscle, between 29.8 and 45.5 μg/kg of muscle during treatment, and depleting to less than the LOQ at 96 hours after withdrawal of medicated feed. Based on these data, dosing with 12 mg of flumequine per kg of body weight as medicated feed, the Committee considered it is unlikely to demonstrate efficacy against bacterial diseases in shrimp (FAO,

2003). In order to address this concern, the 62nd meeting, which re-established the ADI for flumequine, requested by 2006, information on the approved dose for treatment of black tiger shrimp and the results of the residue studies conducted at the recommended dose.

No new information was provided for evaluation by 2006.

Conditions of Use

Flumequine is a fluoroquinolone with antimicrobial activity against Gram-negative organisms. Fluoroquinolones specifically inhibit topoisomerase II (also referred to as DNA gyrase), an enzyme that controls the supercoiling of bacterial DNA by catalyzing the cleavage/reunion of the two strands in the DNA molecule. Binding of fluoroquinolones to DNA gyrase disrupts enzyme activity, resulting in rapid cell death. The bactericidal action of fluoroquinolones is rapid and concentration-dependent. However, activity is inhibited at very high concentrations through direct inhibition of RNA synthesis, which can be antagonized by protein synthesis inhibitors and RNA synthesis inhibitors (Maddison and Watson, 2002).

Flumequine is used in the treatment of enteric infections in food animals and in the treatment of bacterial infections in farmed fish. Flumequine also has limited use in humans for the treatment of urinary tract infections.

METHODS OF ANALYSIS

The 48th meeting of the Committee (FAO, 1998) considered an HPLC method with fluorescence detection for the analysis of flumequine and 7-hydroxyflumequine in the tissues of cattle, pigs, chickens, sheep, and trout. The method had not been properly validated for regulatory purposes. A similar analytical method, which had been validated and was suitable for regulatory purposes, was considered by the 54th meeting.

The 60th meeting of the Committee (FAO, 2003) considered an HPLC method with fluorescence detection for quantifying residues of flumequine in the muscle of black tiger shrimps. The Committee requested that detailed information on a regulatory method including method performance characteristics and method validation be submitted for review.

The present Committee considered two analytical methods for the determination of flumequine residues in shrimps. The first was a method submitted by the government of Thailand (ACFS, 2006) in response to a request by the 60th meeting of the Committee, and re-submitted for consideration by the 66th meeting. The second was a method reported by Pfenning et al. (1996) in the published literature.

Thailand submitted the method of Samuelsen (1990) modified for quantifying flumequine residues in shrimp. The tissue samples are mixed with anhydrous sodium sulfate and extracted twice with ethyl acetate. The sample mixture is centrifuged, and the organic phase removed, filtered and evaporated to dryness under nitrogen at 50-55°C. The residue is reconstituted in mobile phase for HPLC analysis using a C18 column with fluorescence detection (excitation 327 nm; emission 369 nm). A mobile phase of 0.1 M oxalic acid/acetonitrile/methanol (in a ratio, by volume, of 60:30:10) is applied at a flow rate of 1 mL/min.

A single laboratory validation study was conducted to demonstrate method performance. *Linearity* of calibration curves was demonstrated for concentrations of flumequine of 0.5-30 μg/kg (r = 0.9997) and 100-2000 μg/kg (r = 0.9999). *Accuracy* (recovery) was demonstrated at 5, 50 and 500 μg/kg. *Precision* (repeatability within laboratory) was demonstrated at 5, 50

and 500 μg/kg. The numerical performance characteristics for the method are summarized below.

Table 1: Performance characteristics of the liquid chromatographic assay for flumequine residues in shrimp muscle

Performance Characteristic	Shrimp Muscle
LOD (μg/kg)	1
LOQ (μg/kg)	5
Accuracy (%)	80-120
Precision (% CV)	5-7

The method submitted by the government of Thailand has been satisfactorily validated in a single laboratory study and is suitable for regulatory purposes.

Pfenning et al. (1996) reported a method for quantifying flumequine and nalidixic, oxolinic and piromidic acids in salmon and shrimp. The method is the same as that described by Munns et al. (1995) for analyzing residues of flumequine and nalidixic, oxolinic and piromidic acids in catfish. The method determines piromidic acid using liquid chromatography with UV detection, and flumequine, nalidixic acid and oxolinic acid using liquid chromatography with fluorescence detection (325 nm excitation, 360 nm emission). The analysis uses a polymer column in a 46^{o} C oven. The mobile phase is 0.02 M phosphoric acid/acetonitrile/tetrahydrofuran (in a ratio, by volume, of 72:16:12). The identity of all four quinolones present as residues in muscle of salmon and shrimp was confirmed by gas chromatography/mass spectrometry (GC/MS).

Sample preparation involves homogenizing the tissue with acetone, evaporating the acetone and then defatting the extract with hexane and extracting the quinolone residues into chloroform. The extract is further purified by first partitioning into base and then back-extracting from a solution acidified to pH 6.0. The final residue is evaporated to dryness and dissolved in mobile phase for analysis.

Recovery determinations were conducted in composites of shrimp muscle at fortification concentrations of 5, 10, 20, 40 and 80 μg/kg of each quinolone. Average recoveries and relative standard deviations for shrimp, which represent an average of five concentrations for each of the four analytes, were from 81.3 to 91.2% and from 7.3 to 10.7%, respectively. The identity of all four analytes was confirmed in extracts of shrimp muscle fortified at 10 μg/kg by determination of decarboxylated quinolones by GC/MS. Recoveries and relative standard deviations for the determination of flumequine in shrimp muscle are presented in Table 2.

Table 2: Recovery of flumequine from fortified composites of shrimp muscle

Amount added, μg/kg	Mean (n=6), %	SD	RSD, %
5.23	108	11.6	10.8
10.46	94.4	5.82	6.2
20.92	79.4	7.46	9.4
41.84	87.1	3.82	4.4
83.68	87.0	10.5	12.0

No interference resulted from compounds co-eluting with flumequine at its retention time of approximately 12.8 minutes. The analysis of shrimp muscle for flumequine by this method presented no particular problems.

The liquid chromatography method reported by Pfenning et al. (1996) for quantifying flumequine in the muscle of shrimp is validated and suitable for regulatory purposes.

APPRAISAL

The 62nd meeting of the Committee requested information to allow consideration to be given to making the temporary MRL of 500 μg/kg for flumequine in muscle of black tiger shrimp (*Penaeus monodon*) permanent. The results of a single laboratory validation study of the modified Samuelson method were acceptable and the method was considered to be suitable for regulatory purposes. The Committee also considered a published report describing an analytical method for the determination of flumequine in shrimp (Pfenning et al., 1996). This method was similarly validated and considered suitable for regulatory purposes. These data satisfy the provision of new information in respect of a regulatory method as requested by the 62nd meeting.

The 60th meeting evaluated residue depletion studies in shrimp. Based on the available data, dosing with 12 mg of flumequine per kg of body weight as medicated feed is unlikely to demonstrate efficacy against bacterial diseases in shrimp (FAO, 2003). In order to address this concern, the 62nd meeting, which re-established the ADI for flumequine, requested additional information on the approved dose for the treatment of black tiger shrimps and the results of residue studies conducted at the recommended dose. No new information was provided for evaluation.

The present Committee noted the limited availability of approved veterinary drugs for the treatment of aquaculture species. This concern has been addressed by the Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs Without ADI/MRL, which convened in Bangkok in August 2004 (FAO/WHO, 2004). In the absence of data, the Committee could not apply any procedures to extrapolate between species. However, the Committee thought it appropriate to assign the temporary MRL for flumequine to the muscle of all freshwater and marine species of shrimp based on known species similarities.

MAXIMUM RESIDUE LIMITS

The Committee considered the following factors in recommending MRLs:

- An ADI of 0 – 30 μg per kg of body weight based on a toxicological endpoint, which results in a maximum daily intake of 1,800 μg for a 60 kg person.
- Flumequine is the marker residue in shrimp muscle.
- A validated liquid chromatography method with a LOQ of 0.5 μg/kg for flumequine in shrimp muscle is available and suitable for regulatory purposes.
- Information on the approved dose for the treatment of black tiger shrimp and the results of residue studies conducted at the recommended dose had been requested but no new data had been provided.
- The availability of veterinary drugs for the treatment of diseases in aquaculture species is limited.

Based on the above considerations, the Committee recommended maintaining the temporary MRL of 500 μg/kg for muscle of the black tiger shrimp (*Penaeus monodon*) and recommended a temporary MRL to all freshwater and marine species of shrimp. The theoretical maximum daily intake (TMDI) of flumequine residues accounts for 92% of the upper bound of the ADI (FAO, 2000). No suitable data were available to calculate an estimated daily intake (EDI) value. The Committee confirmed its previous request for

information on the approved dose for the treatment of diseases in shrimps and the results of residue depletion studies conducted at the recommended dose. This information is requested by the end of 2008.

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